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(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF NOTTINGHAM [GB/GB]; University Park, Nottingham NG7 2RD (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DUA, Harminder, Singh [GB/GB]; The University of Nottingham, University Park, Nottingham NG7 2RD (GB). HAYNES, Richard, John [GB/GB]; The University of Nottingham, University Park, Nottingham NG7 2RD (GB). TIGHE, Patrick, Jason [GB/GB]; The University of Nottingham, University Park, Nottingham NG7 2RD (GB).

(74) Agent: WOMSLEY, Nicholas; Swindell & Pearson, 48 Friar Gate, Derby DE1 1GY (GB).

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(57) Abstract

The use of defensins in the prevention and therapeutic treatment of ocular infections inclusive of ocular surface and intraocular surface infections. Defensins are short peptide molecules which have antimicrobial and wound healing effects. These have been isolated and purified from human eye tissue and fluids, characterised and identified as important substances for use in the treatment of eye infections.

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DEFENSINS AGAINST OCULAR INFECTION

The present invention relates to ocular infection and particularly but not exclusively to the prevention and therapeutic treatment of ocular infection, inclusive of ocular surface and intraocular infection.

All organs of the body that are in contact with external environments are lined by mucus membrane. The mucus membrane of the eye is the conjunctiva, which together with the corneal epithelium, constitutes the ocular surface. The mucosa of the gut, respiratory and urogenital tract collectively function as the mucosal immune system. Recent evidence indicates that the ocular surface is an integral part of the mucosal immune system. Like the skin, all mucosal surfaces of the body are in constant interaction with environmental microbes and hence vulnerable to infection. The protection of the eyes from microbial attack must have been of paramount importance for the survival of the individual and the preservation of our species. Besides possessing several cell mediated defence modalities in common with other mucus membranes the eye has developed, through evolution, a unique array of protective mechanisms. The preocular tear film contains secretory IgA & IgG, complement components, lactoferrins, lysozyme, beta-lysins, orosomucoid and caeruloplasmin which prevent bacterial adherence and also kill organisms. The antimicrobial activity of tears however is known to be in excess of it's known constituents.

Defensins are naturally occurring peptides that are considered to be among the earliest developed molecular effectors of innate immunity. They are highly conserved molecules being present in many animal classes (mammals, birds, insects and amphibians). Defensins are short peptide molecules with a primary chain length of 29 to 35 amino acids and molecular weight of 3.5 to 4.5 kDa. They are cationic, variably arginine rich and contain six conserved cysteine residues which form three intramolecular disulphide bonds that stabilise a rigid three dimensional ß sheet structure. They function as dimers by creating voltage sensitive channels in the plasma membrane of the target organism. The antimicrobial spectrum of defensins encompasses gram positive

and gram negative bacteria, fungi and viruses (including HIV & HSV). Defensins may also promote a rapid cellular immune response to infection via a chemotactic effect on monocytes. In addition to their antimicrobial actions defensins may accelerate wound healing, by virtue of their mitogenic effect on epithelial cells and fibroblasts.

Two families of mammalian defensins, alpha and beta, have been described. In humans alpha defensins are largely present in neutrophils (alpha defensins 1-4) and in the small intestinal Paneth cells (alpha defensins 5 & 6). Beta defensins have a wider cellular distribution than alpha defensins, beta defensin-1 being expressed by the pancreas, kidney and respiratory epithelium. Recently beta defensin-2 was demonstrated in the skin and bronchial mucosa. No defensin has however been identified and isolated from any ocular tissue in any species.

Many people suffer from ocular infections, such as keratitis and conjunctivitis. In particular, patients who have underlying ocular problems such as dry eyes, conjunctival scarring diseases and nutritional deficiencies; who are critically ill or comatose, or those recovering from ocular surface abrasions or ophthalmic surgery, are prone to eye infections. Contact lens wearers are also particularly prone to ocular surface infections. Individuals who sustain penetrating injury to the eye or undergo intraocular surgery are prone to intraocular infection. At present such infections are treated with antimicrobial drugs such as antibiotics and antiviral and antifungal agents. These however suffer from the disadvantages that they have limited spectrum of activity (e.g. Gram negative bacteria only) they interfere with healing due to ocular surface toxicity and sometimes produce allergic reactions.

According to the present invention there is provided a defensin for use as a therapeutic agent in the treatment of ocular infection.

Preferably the defensin is for use as a therapeutic agent in the treatment of ocular surface and/or intraocular infections.

A plurality of defensins may be used either individually or in combination as such therapeutic agents.

Preferably the defensin(s) is/are purified, recombinant or synthetic.

The defensin(s) is/are preferably purified from human, animal or insect samples, or is/are synthesized.

Preferably the defensin(s) is/are for use by application directly to the site of ocular infection. The defensin(s) may be for use by topical application to the ocular surface or administration by sub-conjunctival or intraocular injection.

The defensin(s) may be alpha and/or beta defensin(s). Preferably the beta defensin is beta defensin 1 and/or beta defensin 2. The alpha defensin is preferably alpha defensin(s) 1-6.

Further according to the present invention there is provided a defensin for use as a preventative agent in the prevention of ocular infection.

Preferably the defensin is for use as a preventative agent in the prevention of ocular surface and/or intraocular infection.

Preferably the defensin is purified, recombinant or synthetic.

The defensin is preferably purified from human, animal or insect samples, or is synthesized. A plurality of defensins may be used either individually or in combination.

Preferably the defensin(s) is/are for use by application directly to the site of ocular infection. The defensin(s) may be for use by topical application to the ocular surface or administration by sub-conjunctival or intraocular injection.

The defensin(s) may be alpha and/or beta defensins. Preferably the beta

defensin(s) is/are beta defensin 1 and/or beta defensin 2. The alpha defensin(s) is/are preferably alpha defensin(s) 1-6.

Further according to the present invention there is provided the use of one or more defensins for the manufacture of a medicament for the treatment of ocular infection.

Preferably the defensin(s) is/are for use in the manufacture of a medicament for the therapeutic treatment of ocular surface and/or intraocular infection.

Preferably the defensin(s) is/are purified, recombinant or synthetic.

The defensin(s) is/are preferably purified from human, animal or insect samples, or are synthesized.

Preferably the defensin(s) is/are for use in the manufacture of a medicament suitable for application directly to the site of ocular infection. The medicament may be for topical application to the ocular surface or administration by sub-conjunctival or intraocular injection.

The defensin(s) may be alpha and/or beta defensins. The beta defensin(s) may be beta defensin 1 and beta defensin 2. The alpha defensin(s) may be any or all of alpha defensins 1-6.

Further according to the present invention there is provided the use of one or more defensins for the manufacture of a medicament for the prevention and/or prophylactic treatment of ocular infection.

The defensin(s) may be for use in the manufacture of a medicament for the prevention of ocular surface infection.

The defensin(s) may be purified, recombinant or synthetic.

The defensin(s) may be purified from human, animal or insect samples, or are synthesized.

The defensin(s) may be for use in the manufacture of a medicament suitable for direct administration to the site of ocular infection. The medicament may be for topical application to the ocular surface or administration by sub-conjunctival or intraocular injection.

The defensin(s) may be alpha and/or beta defensins. The beta defensin(s) may be beta defensin 1 and/or beta defensin 2. The alpha defensin(s) may be one or more of alpha defensins 1-6.

Further according to the present invention there is provided a method of treatment of ocular infection, the method comprising administering one or more defensins to the body.

Preferably the method is for the treatment of ocular surface and/or intraocular infection.

The method may involve the use of one or more purified, recombinant or synthetic defensins.

The defensin(s) may be purified from human, animal or insect samples, or synthesised using known technologies.

The defensin(s) may be administered directly to the site of ocular infection. The defensin(s) may be topically applied to the ocular surface or may be administered by sub-conjunctival or intraocular infection.

The defensin(s) used may be alpha and/or beta defensin(s). The beta defensin(s) may be beta defensin 1 and/or beta defensin 2. The alpha defensin(s) may be one or more of alpha defensins 1-6.

The invention further provides for the use of one or more defensins in the prophylactic treatment of ocular infection, and particularly ocular surface and/or intraocular infection.

The invention further provides one or more isolated and purified defensins for use in the treatment of ocular infection.

The invention still further provides a kit for use in the treatment of ocular infection, the kit comprising one or more defensins and means for administering the defensin(s) to a body for treatment.

An embodiment of the present invention will now be described by way of example only with reference to the accompanying drawings, in which:

Fig. 1 - shows a gel profile of beta defensin-1 expression by RT-PCR. Lane M=100-bp ladder, Lane -ve = negative control sample (DEPC H_2O replacing c-DNA), Lane 1=neutrophils, Lane 2=retina, Lane 3=conjunctiva, Lane 4=cornea, Lane 5=lacrimal gland. Beta defensin-1 primer sequence: 5'-CCC AGT TCC TGA AAT CCT GA-3'; 5'-CAG GTG CCT TGA ATT TTG GT-3'. Product size = 215 bp.

Fig. 2 - shows a gel profile of beta defensin-2 expression by RT-PCR. Lane M=100-bp ladder, Lane 1=skin, Lane 2=conjunctiva, Lane 3=cornea, Lane 4=lacrimal gland, Lane -ve=negative control sample (DEPC H_2O replacing c-DNA). Beta defensin-2 primer sequence: 5'-CCA GCC ATC AGC CAT GAG GGT-3'; 5'-GGA GCC CTT TCT GAA TCC GCA-3'. Product size = 255 bp.

Fig. 3 - shows dot immunoblots of normal human tears showing positive staining for alpha defensin 1-3. Blot A: immunostain with anti defensin 1-3 monoclonal antibody; dot 1=tears, dot 2=non-defensin peptide mixture (negative control). Blot B (negative controls): immunostain as blot A except wash buffer replacing anti defensin 1-3 monoclonal antibody; dot 3=tears, dot 4=non-defensin peptide mixture.

Fig. 4 - shows an immunohistochemistical representation of inflamed conjunctiva showing positive staining for defensin 1-3 in neutrophils in the conjunctival epithelium (arrow) and substantia propria. (Paraffin X 100).

Fig. 5 - shows an immunohistochemistical representation of lacrimal gland showing positive staining for defensin 1-3 in the ductular epithelium (arrows). (Cryosection X 100).

Fig. 6 - shows analysis of beta defensin-1 expression by RT-PCR. Lane M=100-bp ladder. Lane Ciliary body (= post-mortem ciliary body samples) and Lane Positive (= positive control samples, cultured corneal epithelial cells and lacrimal gland samples) RT-PCR confirmed a product at 215 bp, Lane Negative = negative control sample (H_2O replacing c-DNA), Beta defensin-1 primer sequence: 5'CCC AGT TCC TGA AAT CCT GA-3'; 5'-CAG GTG CCT TGA ATT TTG GT-3'. Product size = 215 bp. HPRT PCR reaction in each sample confirms adequate c-DNA levels; and

Fig. 7 - shows dot immunoblots of 7 vitreous and 8 aqueous humour samples showing positive staining for beta defensin-1. Positive control cell culture supernatant containing recombinant human beta defensin-1 and negative control culture medium are clearly positive and negative respectively. Also control duplicate blots processed with the rabbit pre-immune serum in place of the rabbit anti-human beta defensin-1 serum are all negative. APAAP + chemiluminescent substrate.

The present invention provides for the use of one or more defensins in the treatment of ocular infection, and in particular surface and intraocular infections of the eye. Defensins can be used for the manufacture of a medicament for the treatment of such infections when the infection has arisen or prophylactically.

Defensins have been located in the eye and the molecules characterised using the following techniques.

A. Methods for Ocular Surface Defensins

RT-PCR was employed to detect beta defensin 1 & 2 and alpha defensin 5 & 6 messenger-RNA utilising published defensin genomic DNA and messenger RNA sequences to design primers for RT-PCR analysis (15, Gen-Bank database accession numbers X92744, U50930, U50931, Z71389, M96679, M96682, M97925). The only anti-defensin antibody available to date is against alpha defensin 1-3 (DEF 3, Bachem UK Ltd.). This was used for immunohistology and western blotting.

Samples of human corneal and conjunctival epithelium and lacrimal gland were taken from cadaveric eyes donated and consented for transplantation and research. Tear samples were collected from healthy volunteers and aqueous humour samples from patients undergoing cataract surgery. Appropriate consent was obtained from all individuals.

1. Total RNA purification and c-DNA synthesis.

Total RNA was purified from the fresh cell samples using the RNEASY Total RNA Kit (QIAGEN Inc., Chatsworth, CA.) as per manufacturer's instructions. The tissue samples analysed included 3 cornea, 8 conjunctiva, 1 lacrimal gland, 1 sample of neutrophils from peripheral blood, 1 retina, 2 skin and cultured growth arrested bronchial cells.

c-DNA was synthesised directly from total RNA using the "Ready to go" T-Primed First Strand Kit (Pharmacia Biotech) as per manufacturer's instructions. Adequate c-DNA sythesis in each sample was confirmed by employing a Hypoxanthine Phospho Ribosyl Transferase (HPRT) PCR reaction. HPRT is a low level message produced by all living cells, and will be detected by PCR if an adequate amount of c-DNA is present in the sample.

2. PCR

c-DNA (0.5 µl) was added to a mixture (final volume 25 µl) that contained 0.5 µl dNTP mix, 0.25 µl 1% tween, 0.5 µl of both 3' and 5' primers (20 µmol/µl), 1µl of ELONGASE Enzyme Mix (*Taq* and *Pyrococcus species* GB-D thermostable DNA polymerases), 5.0 µl 5x Buffer B and 16.75 µl DEPC water. AmphiTaq Gold DNA polymerase was utilised in alpha defensin 5 & 6 PCR reactions. PCR amplification was performed with an automated thermal cycler for 30 cycles. Beta defensin-1 PCR products was sequenced by an ABI PRISM DNA sequencer.

Beta defensin-1 primers were designed to span the entire intron of beta defensin-1 DNA, thus making contaminant products derived from genomic DNA readily detectable.

The beta defensin-2 primers were based on published m-RNA sequences as the genomic DNA sequence is not currently known. These primers failed to produce an appropriate product when tested with pure genomic DNA, suggesting the primers span an intron. A beta defensin-2 RT-PCR product of the correct size must therefore be derived from the messenger RNA and not genomic DNA. Alpha defensin 5 & 6 intron spanning primers were designed on the basis of published sequences.

3. Immunoblotting

Tear and aqueous humour samples were dot blotted onto a nitrocellulose membrane and allowed to dry. The monoclonal antibody against human alpha defensin 1-3, DEF 3, 1:500, was used as the primary antibody. The secondary antibody, was a rabbit antimouse (DAKO, 1:1000) and the tertiary antibody was mouse APAAP (Alkaline Phosphatase Anti Alkaline Phosphatase, DAKO). 0.05 M Tris buffered saline pH 7.6 was the wash buffer between each antibody step and BCIP/NBT (Sigma Fast) was the substrate. Endogenous alkaline phosphatase activity was blocked with levamisole. Negative control samples, where the primary antibody was replaced with wash buffer only, were run

simultaneously.

4. Immunohistochemistry

Ocular tissue samples were analysed in both paraffin and cryo sections. Normal and pathologically inflamed conjunctival paraffin sections underwent standard immunohistochemistry using DEF 3 as the primary antibody, biotinilated rabbit anti mouse antibody (DAKO 1:500) as the secondary antibody and StreptABC Complex/HRP as the tertiary conjugate. Diaminobenzidine (DAKO) was used as the substrate and 0.05 M Tris buffered saline pH 7.6 was used as the wash buffer. Endogenous peroxidase activity was blocked with methanol + 20vol (6%) hydrogen peroxide solution. Positive and negative controls were run simultaneously.

Cryo samples were transported to the laboratory in saline and immediately orientated on a piece of cucumber, immersed in OCT and snap frozen in liquid nitrogen prior to cryosectioning to 6µm thickness on a Leica CM 1900 cryostat. A standard APAAP staining technique with Fast red substrate (Sigma Fast) was used on lacrimal gland samples (using the antibodies described for immunoblotting above). Neutrophils were purified from citrated whole blood using Polymorphprep (NYCOMED PHARMA AS) as per manufacturer's instructions, fixed to slides by cytospinning and used as positive controls for the DEF 3 antibody.

Results for Ocular Surface Defensins

Beta defensin - 1 RT-PCR of cornea, conjunctiva and lacrimal gland revealed a product at 215 base pairs (Fig. 1). Sequencing of this product revealed an almost exact match with the published sequence of the beta defensin 1 messenger RNA.

Beta defensin-2 RT-PCR revealed a strong signal at 255 base pairs from the cornea sample, a weaker signal from the conjunctiva and no product from

the lacrimal gland (Fig. 2). This result parallels previous experiments which have shown beta defensin-1 but not beta defensin-2 production by the salivary gland, a gland similar in structure to the lacrimal gland. (NB only one lacrimal gland was analysed but adequate c-DNA levels were demonstrated in this sample by the HPRT PCR and the presence of an appropriate product from the beta defensin 1 PCR reaction on the same sample). Sequencing of the corneal RT-PCR product revealed an almost exact match with the published sequence of the beta defensin-2 messenger RNA.

Alpha defensin 5 & 6 RT-PCR did not result in a PCR product in any of the ocular samples tested.

Dot immunoblots of normal human tears showed positive staining for alpha defensin 1-3 (Fig. 3). This suggests defensin 1-3 is free in solution in the tears possibly released from neutrophils. Dot blots of fresh undiluted aqueous humour samples were only weakly positive at the concentrations tested.

Immunohistochemistry of inflamed conjunctiva showed positive staining of masses of neutrophils in the conjunctival substantia propria and epithelium (Fig. 4), however normal un-inflamed conjunctiva did not stain for defensin 1-3. Lacrimal gland ductular epithelium showed staining for defensin 1- 3 (Fig. 5) and high power views revealed staining of intracellular granules within the lining epithelial cells of the ductules.

B. Methods for Intraocular Defensins

RT-PCR. Evidence of defensin production was sought by reverse transriptase polymerase chain reaction (RT-PCR) analysis of seven human post-mortem ciliary body samples donated and consented for transplantation and research, using intron-spanning primers specific for alpha defensin 5 & 6 and beta defensin 1 & 2 messenger-RNA. Ciliary body samples were taken and prepared as soon as possible for total RNA purification using the RNEASY Total RNA Kit (QIAGEN Inc., Chatsworth, CA.) as per manufacturer's instructions. C-

DNA was synthesized direction from total RNA using the "Ready to go" T-Primed First Strand Kit (Pharmacia Biotech, St. Albans, Herts, U.K.) as per manufacturer's instructions. Adequate c-DNA synthesis in each sample was confirmed by including primers to amplify Hypoxanthine Phospho Ribosyl Transferase (HPRT) cDNA in each PCR reaction. Positive control c-DNA samples analyzed included 1 sample from cultured corneal epithelial cells (beta defensin 1 & 2) and 1 sample from lacrimal gland (beta defensin-1 only). PCR reactions were performed as previously described. C-DNA was replaced with water for the negative control reaction. PCR amplification was performed with an automated thermal cycler for 30 cycles using an annealing temperature of 55°C.

Immunoblots. Evidence of actual intraocular defensin peptide was sought by immunoblotting vitreous and aqueous samples using a rabbit anti-human beta defensin-1 polyclonal antibody (HBD-1). Eight aqueous humour samples were obtained with consent from patients undergoing cataract surgery, and seven vitreous humour samples were obtained from patients undergoing pars plana vitrectomy for a variety of pathologies. The vitreous samples were sonicated on ice to reduce viscosity, prior to blotting. Vitreous and aqueous humour samples were blotted directly onto Immobilon-P polyvinylidene difluoride (PVDF) membranes (Millipore (U.K.) Ltd., Watford, U.K.). the blots were fixed for 3h with 10% buffered formalin, washed briefly in Tris buffered saline (TBS: 0.05M, Tris, pH 7.6) and blocked for 30 min in 5% non-fat milk powder in TBS. HBD-1 was used as the primary antibody, with incubation overnight at room temperature. The secondary antibody was swine anti-rabbit serum (DAKO Ltd., Cambridge, U.K.) and the tertiary antibody was rabbit APAAP (alkaline phosphatase anti-alkaline phosphatase, Sigma Chemical Company, Poole, Dorset, U.K.). The blots were placed inside a clear plastic wallet and covered with approximately 1ml of chemiluminescent substrate (Lumi-Phos 530, Boehringer Mannheim, Lewes, East Sussex, U.K.), a piece of Blue Sensitive X-ray film (Genetic Research Instrumentation Ltd., Dunmow, Essex, U.K.) was placed on top and exposed for 1h. Positive and negative control samples were cell culture supernatant containing recombinant human beta defensin-1 and culture medium respectively. Control duplicate blots were

processed with the rabbit pre-immune serum in place of the HBD-1 for comparison.

Results for Intraocular Defensins

Ciliary body RT-PCR for beta defensin-1 revealed a product at 215 base pairs in all 7 samples analyzed. Adequate c-DNA yields were confirmed by HPRT RT-PCR. Positive control cultured corneal epithelial cells and lacrimal gland also revealed a corresponding product at 215 base pairs. The negative control reaction with water replacing c-DNA showed no PCR products (Fig. 6).

Ciliary body RT-PCR for beta defensin-2 and alpha defensin 5 & 6 did not show any appropriate PCR products in the samples tested.

Dot immunoblots of all 7 vitreous and all 8 aqueous humour samples tested showed positive staining with the anti-beta defensin-1 polyclonal antibody (Fig. 7). Positive control cell culture supernatant containing recombinant human beta defensin-1 and negative control culture medium were clearly positive and negative respectively. Also control duplicate blots processed with the rabbit pre-immune serum in place of the rabbit anti-human beta defensin-1 serum were all negative.

Our data suggests that beta defensin-1 is present inside the human eye an dthat the ciliary body contains the messenger-RNA necessary for it's manufacture and secretion into the aqueous humour and vitreous. This is the first evidence of intraocular defensin production and as such presents a new ocular defense mechanism which is relevant to the protection of the vulnerable intraocular environment from microbial invastion.

The human eye therefore appears to innately produce a spectrum of antibiotic defensin peptides, and examples from both alpha and beta defensin subclasses are present in the human eye. It appears that the beta defensins (beta defensin 1 & beta defensin 2) are actually produced by the ocular surface

and that alpha defensins 1-3 are largely released into the ocular surface fluids by resident or passing neutrophils and possibly by secretion from lacrimal ductular epithelia. Alpha defensins 5 & 6 were not detected in the eye, thus on the information currently available, defensin 5 & 6 remain the sole product of Paneth cells.

These results represent the first data revealing defensin production by the human eye. This has enabled development of the present invention.

Defensin molecules can in accordance with the present invention be used as a defence against microbial invasion and therefore the treatment of ocular infection. An understanding of the exact mechanism of production and regulation of defensins at the ocular surface and within the eye will provide further insight into occurrence of microbial corneal ulcers and endophthalmitis, which often leave behind sight threatening sequelae. Historically, it has been well established that breach of corneal epithelial integrity is the most important risk factor in corneal infection. This may be more than just a mechanical effect, with a break in the local defensin barrier also contributing to the susceptibility to infection.

Purified, recombinant or synthetic defensins are ideal therapeutic agents for the eye. They can be administered directly to the site of infection or more general administration can be employed. They could be applied topically or administered by a sub-conjunctival or intraocular injection. The use of one or more defensins, either alone or in combination with other defensins or substances, for the treatment of ocular infection, and the manufacture of medicaments for such treatment both surface and intraocular, represents a considerable breakthrough in the treatment of eye infection. Defensins have a broad spectrum of activity, promote epithelial healing and may be non-antigenic. These characteristics represent considerable advantages over current treatments with antibiotics which have a comparatively limited spectrum of activity (eg. Gram negative bacteria only), which interfere with healing due to ocular surface toxicity and which sometimes produce allergic reactions.

Critically ill and comatose patients, dry eye states and contact lens wearers, are at particular risk of corneal and conjunctival infections, and are some of the clinical situations where such an approach may be of immense benefit.

Whilst endeavouring in the foregoing specification to draw attention to the features of the invention believed to be of particular importance it should be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether of not particular emphasis has been placed thereon.

Claims

1. A defensin for use as a therapeutic agent in the treatment of ocular infection.

2. A defensin according to claim 1, characterised in that the defensin is for use as therapeutic agent in the treatment of ocular surface and/or intraocular infection.

3. A defensin according to claim 1 or claim 2, characterised in that the defensin is used either individually or in combination with one or more other defensins or substances.

4. A defensin according to any of claims 1 to 3, characterised in that the defensin is purified.

5. A defensin according to any of claims 1 to 3, characterised in that the defensin is recombinant.

6. A defensin according to any of claims 1 to 3, characterised in that the defensin is synthetic.

7. A defensin according to any of claims 1 to 4, characterised in that the defensin is purified from one or more of human, animal or insect sample(s).

8. A defensin according to any of the preceding claims, characterised in that the defensin is for use by administration directly to the site of ocular infection.

9. A defensin according to claim 8, characterised in that the defensin is for use by topical application to the ocular surface.

10. A defensin according to any preceding claim, characterised in that the

defensin is for use by administration by sub-conjunctival injection.

11. A defensin according to any preceding claim, characterised in that the defensin is for use by administration by intraocular injection.

12. A defensin according to any of the preceding claims, characterised in that the defensin(s) is/are alpha and/or beta defensin(s).

13. A defensin according to claim 12, characterised in that the beta defensin(s) is/are beta defensin 1 and/or beta defensin 2.

14. A defensin according to claims 12 or 13, characterised in that the alpha defensin(s) is/are alpha defensin(s) 1-6.

15. A defensin for use as a preventative agent in the prevention of ocular infection

16. A defensin according to claim 15, characterised in that the defensin is for use as a preventative agent in the prevention of ocular surface and/or intraocular infection.

17. A defensin according to claims 15 or 16, characterised in that the defensin is purified.

18. A defensin according to claims 15 or 16, characterised in that the defensin is recombinant.

19. A defensin according to claims 15 or 16, characterised in that the defensin is synthetic.

20. A defensin according to claims 15 to 17, characterised in that the defensin is purified from one or more of human, animal or insect sample(s).

21. A defensin according to claims 15 to 19, characterised in that the defensin is used either individually or in combination with one or more other defensins or substances.

22. A defensin according to any of claims 15 to 20, characterised in that the defensin(s) is/are for use by administration directly to the site of ocular infection.

23. A defensin according to claim 22, characterised in that the defensin(s) is/are for use by topical application to the ocular surface.

24. A defensin according to claim 22, characterised in that the defensin(s) is/are for use by administration by sub-conjunctival injection.

25. A defensin according to claim 22, characterised in that the defensin(s) is/are for use by administration by intraocular injection.

26. A defensin according to any of claims 15 to 25, characterised in that the defensin(s) is/are alpha and/or beta defensin(s).

27. A defensin according to claim 26, characterised in that the beta defensin(s) is/are beta defensin 1 and/or beta defensin 2.

28. A defensin according to claims 26 or 27, characterised in that the alpha defensin(s) is/are alpha defensin(s) 1-6.

29. Use of one or more defensins for the manufacture of a medicament for the treatment of ocular infection.

30. Use of one or more defensins according to claim 29, characterised in that the defensin(s) is/are for use in the manufacture of a medicament for the therapeutic treatment of ocular surface and/or intraocular infection.

31. Use of one or more defensins according to claims 29 or 30, characterised in that the defensin(s) is/are purified.

32. Use of one or more defensins according to claims 29 or 30, characterised

in that the defensin(s) is/are recombinant.

33. Use of one or more defensins according to claims 29 or 30, characterised in that the or any of the defensin(s) is/are synthetic.

34. Use of one or more defensins according to claims 29 to 31, characterised in that the or any of the defensin(s) is/are purified from human, animal or insect samples.

35. Use of one or more defensins according to claims 29 to 34, characterised in that the defensin(s) is/are for use in the manufacture of a medicament suitable for administration directly to the site of ocular infection.

36. Use of one or more defensins according to claim 35, characterised in that the medicament is for topical application to the ocular surface.

37. Use of one or more defensins according to any of claims 29 to 35, characterised in that the medicament is for administration by sub-conjunctival injection.

38. Use of one or more defensins according to any of claims 29 to 35, characterised in that the medicament is for administration by intraocular injection.

39. Use of one or more defensins according to any of claims 29 to 38, characterised in that the defensin(s) is/are alpha and/or beta defensin(s).

40. Use of one or more defensins according to claim 39, characterised in that

the beta defensin(s) is/are beta defensin 1 and/or beta defensin 2.

41. Use of one or more defensins according to claims 39 or 40, characterised in that the alpha defensin(s) is/are any or all of alpha defensin(s) 1-6.

42. Use of one or more defensins for the manufacture of a medicament for the prevention and/or prophylactic treatment of ocular infection.

43. Use of one or more defensins according to claim 42, characterised in that the defensin(s) is/are for use in the manufacture of a medicament for the prevention of ocular surface and/or intraocular infection.

44. Use of one or more defensins according to claims 42 or 43, characterised in that the or any of the defensin(s) is/are purified.

45. Use of one or more defensins according to claims 42 or 43, characterised in that the or any of the defensin(s) is/are recombinant.

46. Use of one or more defensins according to claims 42 or 43, characterised in that the or any of the defensin(s) is/are synthetic.

47. Use of one or more defensins according to any of claims 42 to 44, characterised in that the or any of the defensin(s) is/are purified from human, animal or insect samples.

48. Use of one or more defensins according to any of claims 42 to 47, characterised in that the defensin(s) is/are for use in the manufacture of a medicament suitable for direct administration to the site of ocular infection.

49. Use of one or more defensins according to claim 48, characterised in that the medicament is for topical application to the ocular surface.

50. Use of one or more defensins according to any of claims 42 to 48,

characterised in that the medicament is for administration by sub-conjunctival injection.

51. Use of one or more defensins according to any of claims 42 to 48, characterised in that the medicament is for administration by intraocular injection.

52. Use of one or more defensins according to any of claims 42 to 51, characterised in that the defensin(s) is/are alpha and/or beta defensin(s).

53. Use of one or more defensins according to claim 52, characterised in that the beta defensin(s) is/are beta defensin 1 and/or beta defensin 2.

54. Use of one or more defensins according to claim 52 or 53, characterised in that the alpha defensin(s) is/are one or more of alpha defensin(s) 1-6.

55. A method of treatment of ocular infection, the method comprising administering one or more defensins to the body.

56. A method according to claim 55, characterised in that the method is for the treatment of ocular surface and/or intraocular infection.

57. A method according to claims 55 or 56, characterised in that the method may involve the use of one or more purified defensins.

58. A method according to claim 55 or 57, characterised in that the method may involve the use of one or more recombinant defensins.

59. A method according to claim 55 or 58, characterised in that the method may involve the use of one or more synthetic defensins.

60. A method according to any of claims 55 to 59, characterised in that the or any of the defensin(s) is/are purified from human, animal or insect samples

using known techniques.

61. A method according to any of claims 55 to 59, characterised in that the defensin(s) is/are synthesised using known techniques.

62. A method according to any of claims 55 to 61, characterised in that the defensin(s) is/are administered directly to the site of ocular infection.

63. A method according to claim 62, characterised in that the defensin(s) is/are topically applied to the ocular surface.

64. A method according to any of claims 55 to 63, characterised in that the defensin(s) is/are administered by sub-conjunctival infection.

65. A method according to any of claims 55 to 63, characterised in that the defensin(s) is/are administered by intraocular injection.

66. A method according to any of claims 55 to 65, characterised in that the defensin(s) used is/are alpha and/or beta defensin(s).

67. A method according to claim 66, characterised in that the beta defensin(s) is/are beta defensin 1 and/or beta defensin 2.

68. A method according to claims 66 or 67, characterised in that the alpha defensin(s) is/are one or more alpha defensins 1-6.

69. Use of one or more defensins in the prophylactic treatment of ocular infection.

70. Use of one or more defensins according to claim 69, characterised in that the defensin(s) is/are used in the prophylactic treatment of ocular surface and/or intraocular infection.

71. One or more isolated and purified defensins for use in the treatment of ocular infection

72. A kit for use in the treatment of ocular infection, the kit comprising one or more defensins and means for administering the defensin(s) to a body for treatment.

73. A defensin for use as a therapeutic agent in the treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

74. A defensin for use as a preventative agent in the prevention of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

75. Use of one or more defensins for the manufacture of a medicament for the treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

76. Use of one or more defensins for the manufacture of a medicament for the prevention and/or prophylactic treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

77. A method of treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

78. Use of one or more defensins in the prophylactic treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

79. One or more isolated and purified defensins for use in the treatment of ocular infection substantially as hereinbefore described with reference to the

accompanying examples and drawings.

80. A kit for use in the treatment of ocular infection substantially as hereinbefore described with reference to the accompanying examples and drawings.

81. Any novel subject matter or combination including novel subject matter disclosed herein, whether or not within the scope of or relating to the same invention as any of the preceding claims.

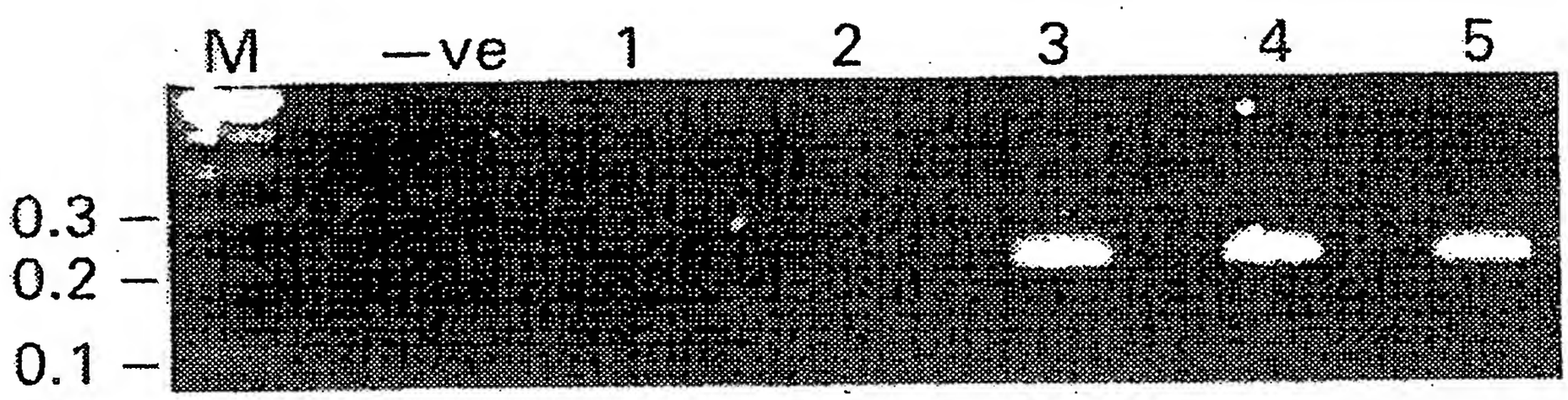


FIG. 1

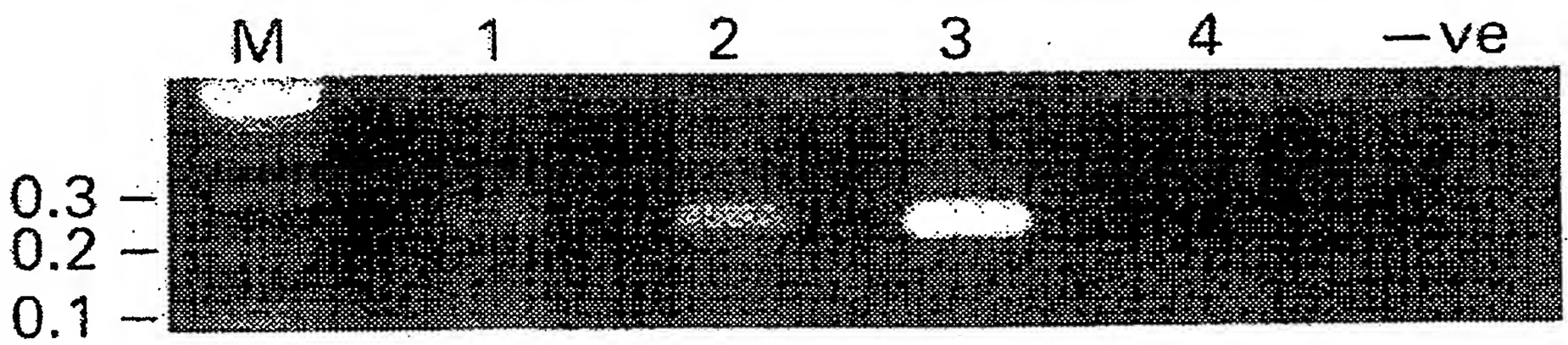


FIG. 2

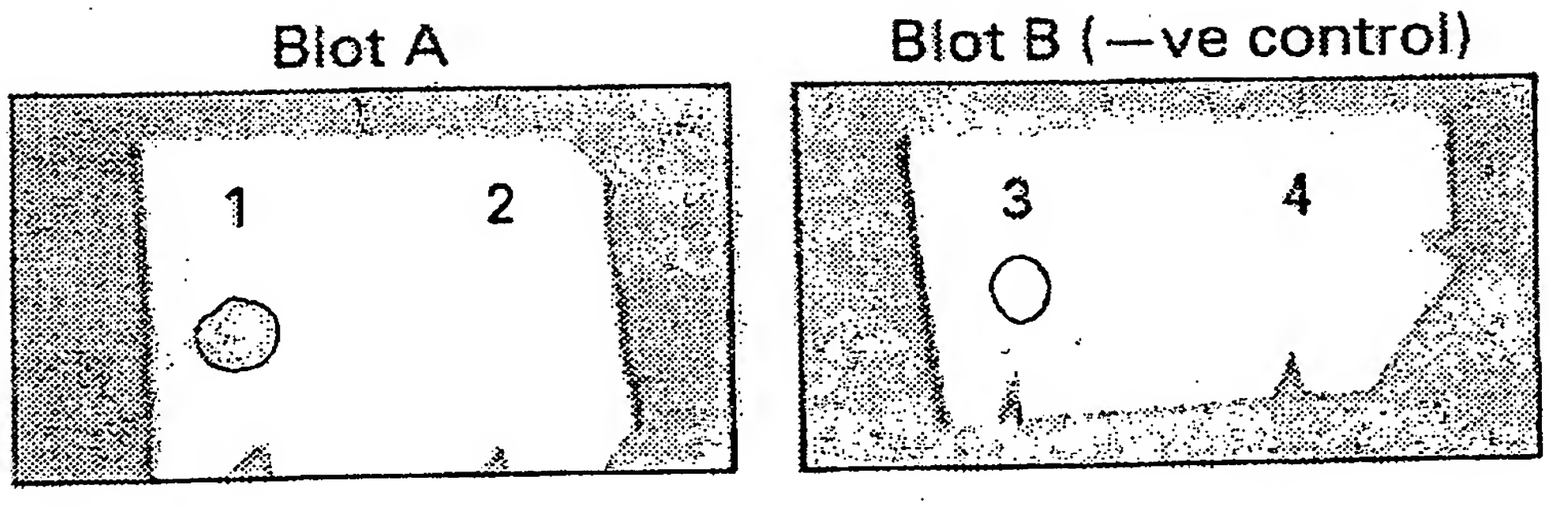


FIG. 3

Fig 4

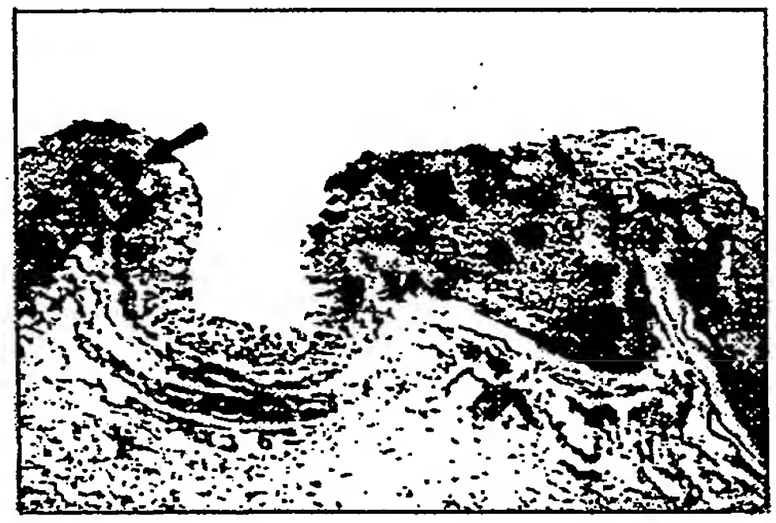
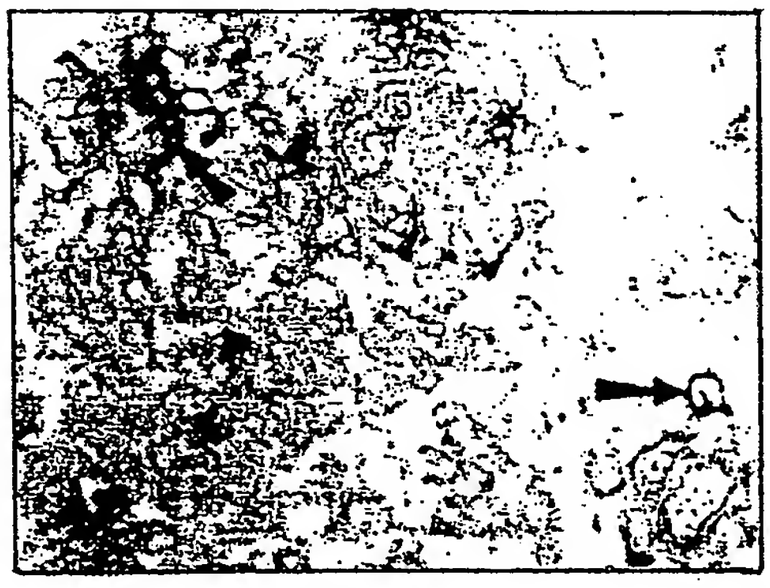


Fig 5



M Ciliary body Positive Negative

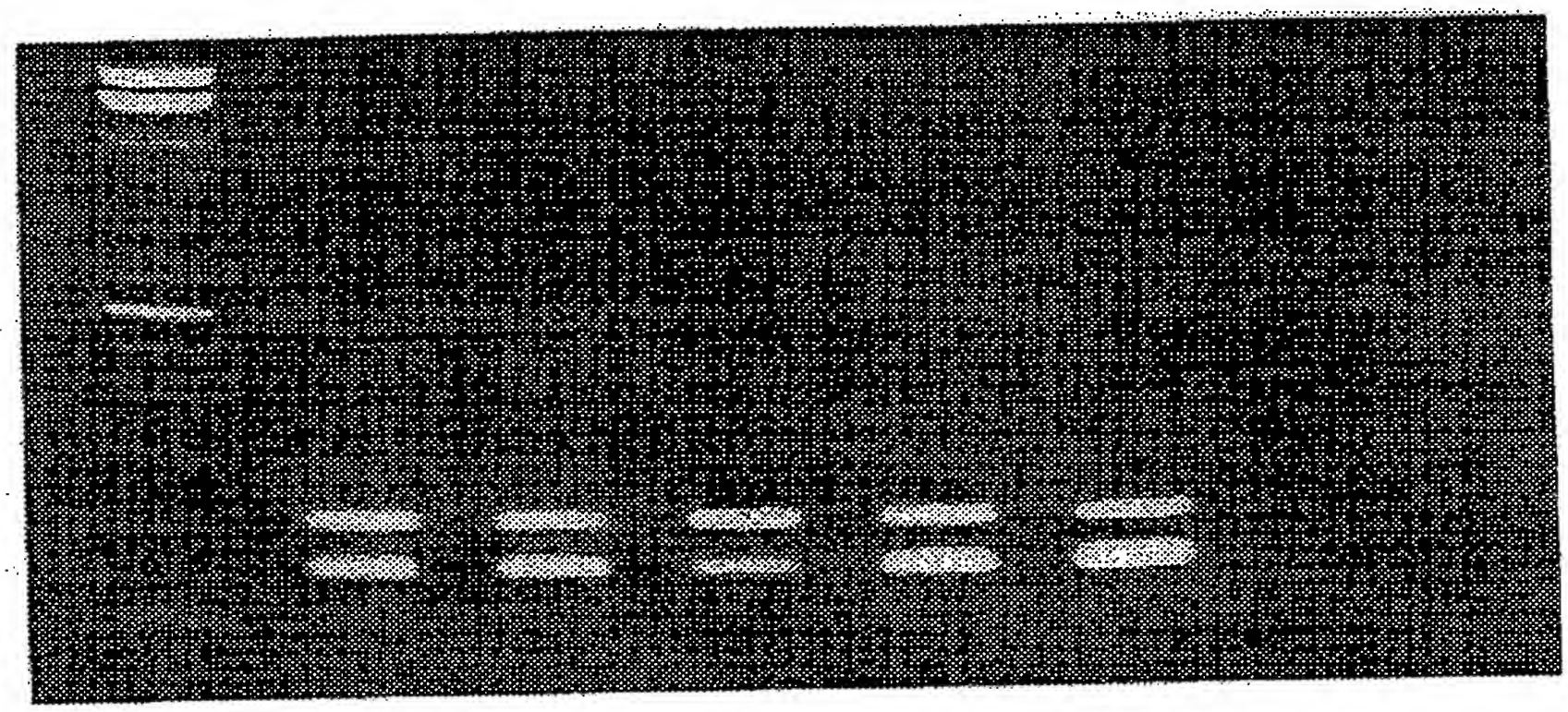


Fig.6

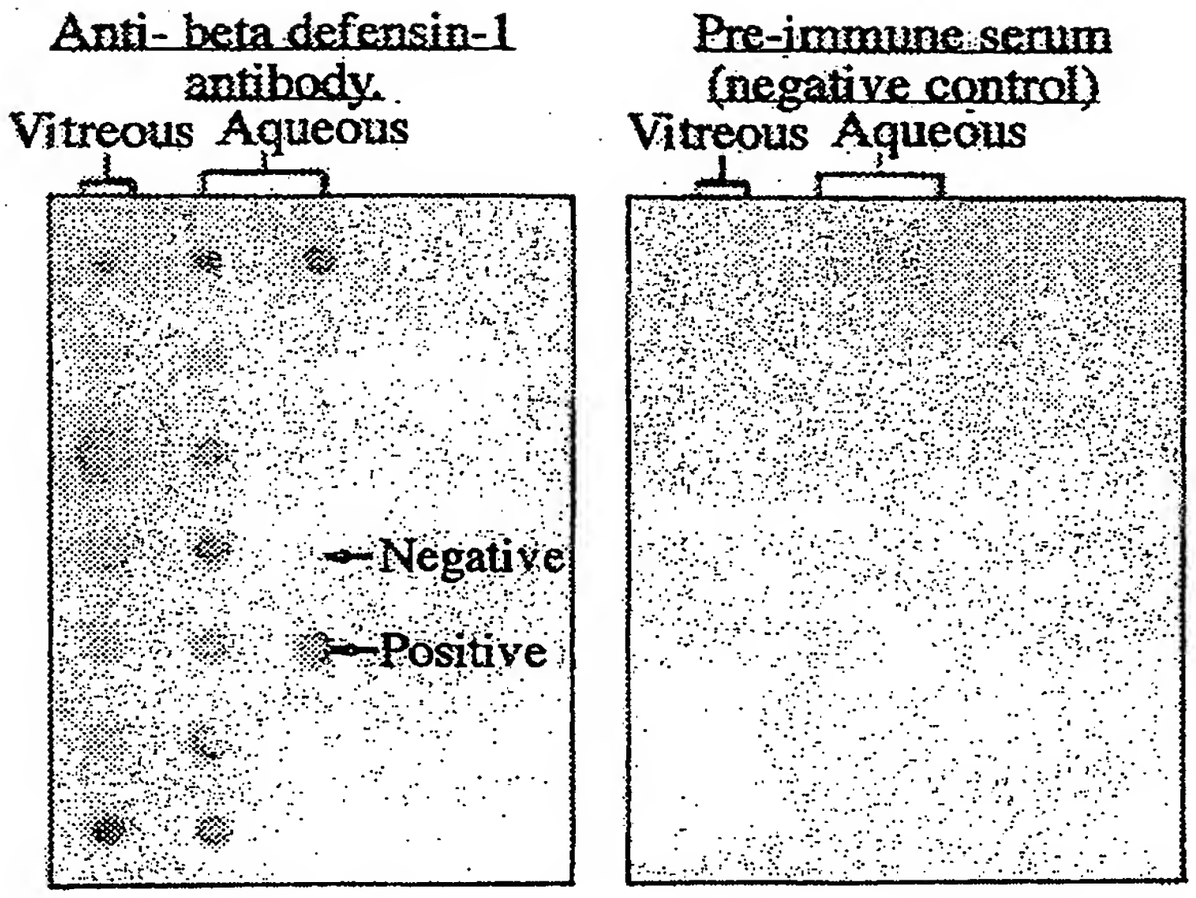

Anti- beta defensin-1 antibody.
Vitreous Aqueous
Negative
Positive
Pre-immune serum (negative control)
Vitreous Aqueous

FIG. 1

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 99/02591

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 242 902 A (MURPHY CHRISTOPHER J ET AL) 7 September 1993 (1993-09-07)	1-12, 14-26, 28-39, 41-52, 54-66, 68-81
Y	column 2, line 63 -column 3, line 18 column 4, line 29 - line 36; claims	13,27, 40,53,67

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 1999	12/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Teyssier, B

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int[illegible]ional Application No
PCT/GB 99/02591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 16066 A (MAGAININ SCIENCES INC) 31 October 1991 (1991-10-31) page 1, line 4 - line 9 page 19, line 25 - line 33	1-12, 14-26, 28-39, 41-52, 54-66, 68-81
Y	---	13,27, 40,53,67
Y	DE 44 27 531 A (FORSSMANN WOLF GEORG) 8 February 1996 (1996-02-08) page 2; claims 1-6,11,12	13,27, 40,53,67
A	---	1-81
	HANCOCK R E W ET AL: "Cationic peptides: a new source of antibiotics" TRENDS IN BIOTECHNOLOGY, vol. 16, no. 2, pages 82-88, XP004107047 ISSN: 0167-7799 page 83, section "Vertebrate alpha and beta defensins"	
P,X	---	1-81
	HAYNES R J ET AL: "Innate defence of the eye by antimicrobial defensin peptides" THE LANCET, vol. 352, 8 August 1998 (1998-08-08), pages 451-452, XP002126263 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 55-68 and 77 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 81
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 81

Present claim 81 relates to an extremely large number of possible compounds/products/apparatus/methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products/apparatus/methods claimed. In the present case, the claim so lacks support and clarity that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claim which appear to be supported and disclosed, namely claims 1-80.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]ional Application No
PCT/GB 99/02591

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5242902 A	07-09-1993	NONE	
WO 9116066 A	31-10-1991	CA 2040510 A	24-10-1991
		EP 0528861 A	03-03-1993
		JP 5509295 T	22-12-1993
		US 5221664 A	22-06-1993
DE 4427531 A	08-02-1996	NONE	